dispensed simultaneously or sequentially to different locations on the bottom substrate (again, through the ports in the top plate). That is, the channels put the exit port of the blister (however configured, as outlined below) in fluid communication with the appropriate electrowetting location on the substrate.

[0179] In addition to the blisters of the LRM, the LRM comprises a sample entry port to introduce a sample into the cartridge. This generally is configured to receive a standard pipette tip, used to add the appropriate volume of sample to the cartridge. Either the LRM or the housing, discussed below, contains a sealing mechanism such as a latched cover to both seal the cartridge so as not to introduce any contaminants as well as prevent the escape of biological materials. In general, as depicted in the figures, it is the housing that comprises the latched sealing cover.

[0180] The LRM optionally includes capture beads (e.g. magnetic capture beads) which can be dispensed into the chamber within the electrowetting grid. The beads are the preferred mechanism of transfer between the LRM and the PCB. Typically, beads bind DNA/RNA in the LRM, are washed in the LRM, and then transferred to the PCB with a volume of wash buffer (e.g., 100-200 μl), where electrowetting facilitates elution of the DNA/RNA in a small volume. Once delivered to the cartridge, the beads are collected over an area of the electrowetting grid which has a magnet applied underneath from the bottom part of the bay. Beads with elution buffer are subsequently subjected to electrowetting to mix for elution. The elution volume of a few microliters would be difficult to achieve on the LRM or in any other non-electrowetting setup.

[0181] In some embodiments, the LRM can contain pumps to facilitate movement of the reagents and/or sample from the LRM to the bottom substrate, although in general the "no moving parts" principle dictates that these pumps, if necessary, would be off chip. A number of microfluidic pumps are known. In a typical embodiment, however, the pump is not contained in the consumable. A notable exception is where a pump, such as a pair of umbrella valves or other type of one-way valve, is contained in the LRM but driven by an external mechanism.

[0182] In some optional embodiments, some of the ports and/or channels comprise one or more valve(s) to control the flow of reagents and/or samples. In many cases, one way valves find use, such that a fluid is moved from the LRM into the chamber volume and cannot backflow or return to the LRM. Generically, these include normally-open valves and normally-closed valves. There are a variety of one way valves known, e.g., duck bill valves.

[0183] In some optional embodiments, the LRM/top plate components contain one or more vent(s) to reduce air bubbles, which are particularly undesirable in the detection zone, and can be formed in some instances during the thermocycling. In these embodiments, the vent(s) can simply be holes or vias that connect certain areas of the reaction chamber with a reservoir in the LRM. Alternatively, the vents may use valves (particularly one way valves), or can be coated or filled with materials that allow air to pass but prevent liquid exit (such as GORTEX® or other hydrophobic materials). In a typical embodiment, a Teflon® membrane with about 0.2 μm pores can be used. Generally, any sufficiently hydrophobic material with pores roughly in the 0.1-1 micron range could be used.

[0184] In addition to the deformable blisters used to dispense liquids during the assay protocols, the LRM can also comprise one or more chambers that are generally not deformable but are used for specific sample or reagent handling. For example, as outlined herein, the LRM can also optionally comprise one or more mixing chambers that facilitate mixing of the sample with reagents. For example, as described herein, chamber(s) containing impeller(s) can be used, particularly to grind up solid samples, maximize exposure of sample to capture beads, mix sample with chemical lysis buffer, mix magnetic beads with binding buffer (typically magnetic beads cannot be stored in their binding buffer), etc. Alternatively, mixing can be done within the reaction chamber by moving the sample droplets back and forth between pads, and/or splitting and merging sample droplets to maximize mixing. In some embodiments, one or more chambers of the LRM. Similarly, the LRM can comprise one or more waste chambers in which to place excess or used fluids.

[0185] In some optional embodiments, the LRM comprises one or more opening(s) that allow one or more optical sensor (s) to monitor the progress of reagents and sample through the LRM, e.g., to detect a transition point between air and liquid when air is employed to motivate the sample and/or a liquid reagent. The sensor itself is preferably located in the bay. The openings provide the sensor with optical access to "see" into the LRM. Other fluid sensors could be used, notable inductive, capacitive, resistive, or other electrical sensors.

[0186] In some embodiments, the LRM can include one or more porous filters to remove particulates from the sample prior to downstream processing. For example, there may be a filter between the capture beads and the elution chamber, such that the eluent flows through a filter to remove particulates prior to the amplification step. The filter is preferably located as early as possible in the process flow to keep particulate matter out of system, or immediately after lysis to remove anything that did not get lysed.

[0187] Particular and specific embodiments of the LRM utilize deformable fluid vessels, or blisters, as described in more detail below.

Manipulation of Deformable Fluid Vessels

[0188] In the present invention, one LRM embodiment that finds use in a variety of systems and assays relies on the use of deformable fluid vessels, sometimes referred to herein as "blisters" or "blister packs". There are a number of configurations and embodiments, as generally outlined in FIGS. **1-19**.

[0189] An actuator mechanism for compressing deformable fluid vessels—such as blisters on a liquid reagent module—embodying aspects of the present invention is shown at reference number **50** in FIG. **2**. The actuator mechanism **50** will reside in the top part of the bay and may include an articulated blister actuator platen assembly **52** and a sliding actuator plate **66**. The sliding actuator plate **66** is configured to be movable in a direction that is generally parallel to the plane of the liquid reagent module—horizontally in the illustrated embodiment—and may be driven by a linear actuator, a rack and pinion, a belt drive, or other suitable motive means. Sliding actuator plate **66**, in the illustrated embodiment, has V-shaped edges **76** that are supported in four V-rollers **74** to accommodate movement of the plate **66** in opposite rectilinear directions, while holding the sliding actuator plate **66** at a fixed spacing from the actuator platen assembly **52**. Other